ULTRASTRUCTURE OF THE PROTEGULUM OF SOME ACROTRETIDE BRACHIOPODS

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ABSTRACT. Electron microscopic studies of the exteriors of a number of acrotretacean genera show that their protegula were ornamented by shallow pits, usually with a coarser set, about 2 μm in diameter, partially or completely segregated from one another by groups of smaller ones about 300 nm across. The pattern is identical with the mould of a bubble raft, and consideration of the structure of the periostracum of living terebratellaceans suggests that the pits ornamenting the acrotretacean protegulum are the moulds of a highly vesicular periostracum up to 3 μm thick with a thin (10 nm) inner sealing membrane. Such a periostracum would have afforded larvae extra buoyancy immediately prior to their settlement on the substrate. The absence of pit ornamentation from the adult shell is believed to indicate the development of an inner sealing membrane which was sufficiently thick to mask the vesicular nature of the adult periostracum. Nothing comparable with this ornamentation is known in other Acrotretida, although the protegulum of *Eoconulus*, which may be an aberrant craniacean, included a mineral mesh of regular, alternating, circular holes about 8 μm in diameter which have been attributed to differential secretion beneath a non-vesicular periostracum comparable with that covering living *Crania*.

THE brachiopod shell usually bears traces of important ontogenetic changes in composition, structure, and secretory rates because resorption by the mantle is restricted to internal surfaces of the valves. In Recent species, the topography of external surfaces reflects growth variation at an ultrastructural level, and although the exteriors of calcareous-shelled fossils are usually masked by adherent micritic calcite of diagenetic origin, those of extinct chitino-phosphatic inarticulate brachiopods can normally be freed of such accretionary deposits by differential etching in acetic acid. The diagenetic processes which allow for this fortunate circumstance are not well understood. Presumably, the chitin and protein of the periostracum and of those layers alternating with apatite within the shell, break down into organic complexes which persist as continuous sheets to preclude any gross recrystallisation of the calcium phosphate, either in continuity with, or independently of, the rock matrix. Consequently even ultramicroscopic details of the topography of the external mineral or organic surfaces of most fossil inarticulates, may, in favourable conditions, be preserved for examination. This fact became evident in a survey of some Acrotretida under the scanning electron microscope. Except for Recent specimens of *Crania* and *Discinisca*, all acrotretide shells examined have been etched out of a variety of sediments; yet many show, to differing degrees of perfection, a previously unsuspected ornamentation of pits on the external surface of the protegulum. As will be shown, the pits may be interpreted as moulds of the undersurface of a distinctive type of periostracum which has its counterpart in living Terebratulida and which may even have contributed to the buoyancy of acrotretide larvae during the planktonic phase of their existence.

In the text, references are made to the protegular structure of a number of undescribed species. Formal systematic accounts of these will be published in due course by G. Biernat, who is currently investigating the Ordovician Inarticulata of Poland.

Materials and methods. The section of the periostracum illustrated in this paper was prepared by fixing a young *Waltonia inconspicua* (Sow.) in 3% gluteraldehyde made up in 3% sodium chloride buffered

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to pH 7·2 with phosphate buffer. Material was subsequently decalcified in 5·5% EDTA, washed with sucrose, and treated for 1 hour with 1% osmic acid; all these solutions were buffered to pH 7·2 with phosphate buffer. Following dehydration, the specimen was embedded in Epon-Araldite resin and the sections stained with alcoholic uranyl acetate and aqueous lead citrate. Surfaces of dried periostracum were replicated for the transmission electron microscope by casting them in cellulose acetate strips which, before being dissolved away, were shadowed with gold-palladium at 1 to 1 and coated in carbon. Shell surfaces and sections studied under the 'Stereoscan' scanning electron microscope, were coated with gold-palladium.

THE PROTEGULUM

The protegulum is usually described as the first-formed shell of the brachiopod, secreted simultaneously over the entire surfaces of both mantles in the larval or early post-larval stages of development. The precise sequence of events leading to its appearance, however, is poorly known. Percival (1944, pp. 9–10) reported that, subsequent to the attachment of the larva of *Waltonia inconspicua* (Sow.) and during the last stages of enclosure of the anterior lobe by the reversing mantle, there is 'clear evidence of the formation of a hard shell'. At this stage in development the shell is about 120 μm long and consists of an outer periostracum and an inner layer of calcite crystallites. The protegulum of *Notosaria nigricans* (Sow.) has the same two-layered structure and is also secreted at about the same time after the settling of the larva on the substrate (Percival 1960, p. 448). In contrast, the protegulum of living inarticulates is, as far as is known, secreted before larval attachment. According to Yatsu (1902, p. 31) the mantle lobes of the larva of *Lingula unguis* (Linneus) are differentiated from a rudimentary ring-like flap when the larva is free-swimming. They then secrete a thin chitinous shell in one piece which is folded across the posterior margin to form a pair of semicircular valves about 140 μm in radius. Similarly, the protegulum is known to have been secreted in the youngest free-swimming larva of *Pelagodiscus* or any other discinid yet recovered (Chuang 1968, p. 265) and to have been present in the earliest identifiable individuals of *Crania* and *Discinisca* which were already attached to the substrate (Williams and Rowell *in* Williams *et al.* 1965, p. H50). Despite this difference in the timing of the deposition of the protegulum relative to the free-swimming stage in brachiopod ontogeny, correlation of the skeletal successions shows that the secretory regimes of the outer epithelium of the rudimentary mantle lobes follows the same sequence in widely different species. Studies of newly formed cells at the mantle edge of articulate brachiopods (Williams 1968) as well as unpublished observations of the mantle generative zones in *Lingula* and *Glottidia*, suggest that the first-formed cover of the outer epithelium is likely to have been always a mucopolysaccharide. Such a cover is probably maintained by continuous exudation over the entire surface of the larva until the outer and pedicle epithelia become differentiated and secrete the first persistent layer of the exoskeleton. Over the outer epithelium of the mantle lobes, this layer, the periostracum, is invariably composed of protein and/or chitin. The first-formed periostracum is exuded very rapidly and may immediately become the seeding sheet for deposits of calcite or apatite crystallites. Consequently it is always possible for the outer surface of such a mineral layer to form a mould of the topography of the inner periostracal surface, especially when that surface is only a sealing membrane about 10 nm thick.

The structure of the fossil protegulum is invariably incomplete and its precise boundaries may not be determinable even on the umbones of well-preserved shells. In general, all external covers of organic origin are lost from the surfaces of fossil shells through the processes of weathering and diagenesis, and only the inner mineral layer remains. Indeed in those specimens where the protegulum was exclusively organic in composition, a mould could be the only surviving trace of the structure and this condition may be characteristic of some of the species discussed below. There is no infallible guide for determining the limits of the protegulum on adult shells. Protegula are known to range from less than 100 to more than 1000 μm in length, and from semi-elliptical to subcircular in outline. The outline is usually accentuated as a distinct step in the general profile of the umbones so that protegula appear to sit on the apices of the valves as extra-skeletal pieces. More importantly, no growth-lines should occur on the surface of a protegulum because it is secreted simultaneously over the entire mantle lobe. This criterion and the expectation that the protegular outline is normally accentuated have been used for the identification of acrotretide protegula.

THE ACROTRETACEAN PROTEGULUM

The fabric of the protegulum of an undescribed species of *Torynelasma* may be taken as typifying that of acrotretacean protegula in general. The ventral protegulum, which is about 100 μm wide (Pl. 98, fig. 1), is not ornamented by raised concentric ridges characteristic of the adult shell, but by a series of shallow circular to elliptical pits (Pl. 98, fig. 2). The pits vary in diameter by more than a factor of ten, although they actually fall into two grades. The coarser pits range from 2 to 4·5 μm in diameter and are separated from one another by ridges, about 350 nm wide, which swell out into flattened areas, up to 4 μm wide, intervening between groups of coarser pits. These flattened areas, as well as some of the ridges, bear the finer grade of pits which are about 350 nm in diameter. All pits are more or less flat-bottomed and about one-tenth as deep as the maximum diameter. The entire fabric of pits and ridges is preserved in apatite crystallites (each about 175 nm in diameter) which are stacked normal to the surface. The junction between the protegulum and later shell is abrupt; within a micron of being fully developed the pits pass into very shallow dimples which in turn give way to the first concentric ridges ornamenting the surface of adult shells (compare Pl. 98, fig. 5). The pits are impressed on the external surface of a mineral layer or lamina, about 2 μm thick, which is underlain by up to six laminae of comparable thickness (Pl. 98, fig. 3). These laminae are composed of apatite crystallites stacked more or less normal to their external and internal surfaces. They are separated from one another by gaps about 170 nm wide which were probably occupied by organic sheets. The entire fabric is reminiscent of laminar deposition in the craniaceans (Williams and Wright, in press), although the mineral constituent is calcium phosphate not calcium carbonate, and the accretion of laminae may involve continuous vertical growth of densely distributed apatite seeds instead of the spiral growth of calcite rhombohedra.

Sampling among other members of the Acrotretacea suggests that the protegular pit pattern of *Torynelasma* is characteristic of the superfamily. Sixteen species belonging to twelve genera, ranging in age from Middle Cambrian to late Ordovician, have provided the information given in Table 1. In addition, the protegular surface of *Ceratreta hebes* Bell from the Upper Cambrian Dry Creek Shale of Montana showed identifiable traces of pits although they were too poorly preserved to be accurately measured or figured. In all species the protegulum, which varied in maximum diameter from 90 to 135 μm, was ornamented by pits fundamentally the same in structure and arrangement as those of *Torynelasma*. There is a variation in the size and distribution of pits, which may ultimately prove to be of systematic value. Thus the pits ornamenting the surface of the protegula of *Conotreta* (Pl. 99, fig. 1), *Linnarssonella* (Pl. 99, fig. 2), *Myotreta* (Pl. 98, figs. 5, 6), and *Rhysotreta* (Pl. 98, fig. 4) are comparable in size range with those

TABLE 1. The ranges of larger pits (*a*) and the diameter of smaller pits (*b*) forming the ornamentation on the protegula of the listed species. The horizons and locations of the specimens providing these data are given in descriptions of plates except for the unfigured *Scaphelasma septatum* Cooper and *Torynelasma toryniferum* Cooper, both of which were represented by topotypic material from the mid-Ordovician Pratt Ferry Formation, Pratt Ferry, Alabama.

	(*a*) *larger pits* (μm)	(*b*) *smaller pits* (nm)
Angulotreta postapicalis Palmer	about 1·5	—
Apsotreta expansa Palmer	1·0–2·3	—
Conotreta depressa Cooper	1·25–2·5	450
Curticia minuta Bell	0·8–3·2	320
Ephippelasma sp.	0·8–1·69	600
Linnarssonella girtyi Walcott	1·9–3·8	—
Myotreta cf. *crassa* Goryansky	2·0–4·6	500
Prototreta sp.	0·96–1·6	320
Rhysotreta corrugata Cooper	1·55–3·1	300
Scaphelasma septatum Cooper	0·9–3·0	300
Scaphelasma sp.	1·1–2·3	700
Spondylotreta concentrica Cooper	0·8–1·7	150
Spondylotreta sp.	0·7–2·0	—
Torynelasma sp.	2·2–4·5	360
Torynelasma toryniferum Cooper	1·2–2·3	380

of *Torynelasma*, whereas the coarser grades found in *Ephippelasma* (Pl. 99, fig. 3), *Prototreta* (Pl. 99, fig. 4), *Scaphelasma* (Pl. 99, fig. 5), and *Spondylotreta* (Pl. 100, fig. 3) are significantly smaller. There are also differences in the frequency distribution of the coarser pits, which are more closely packed together in *Conotreta*, *Myotreta*, and *Prototreta*, so that intervening flattened areas bearing clusters of fine pits, as in *Torynelasma*, are comparatively rare. In *Prototreta*, the fine pits appear to have been obliterated in

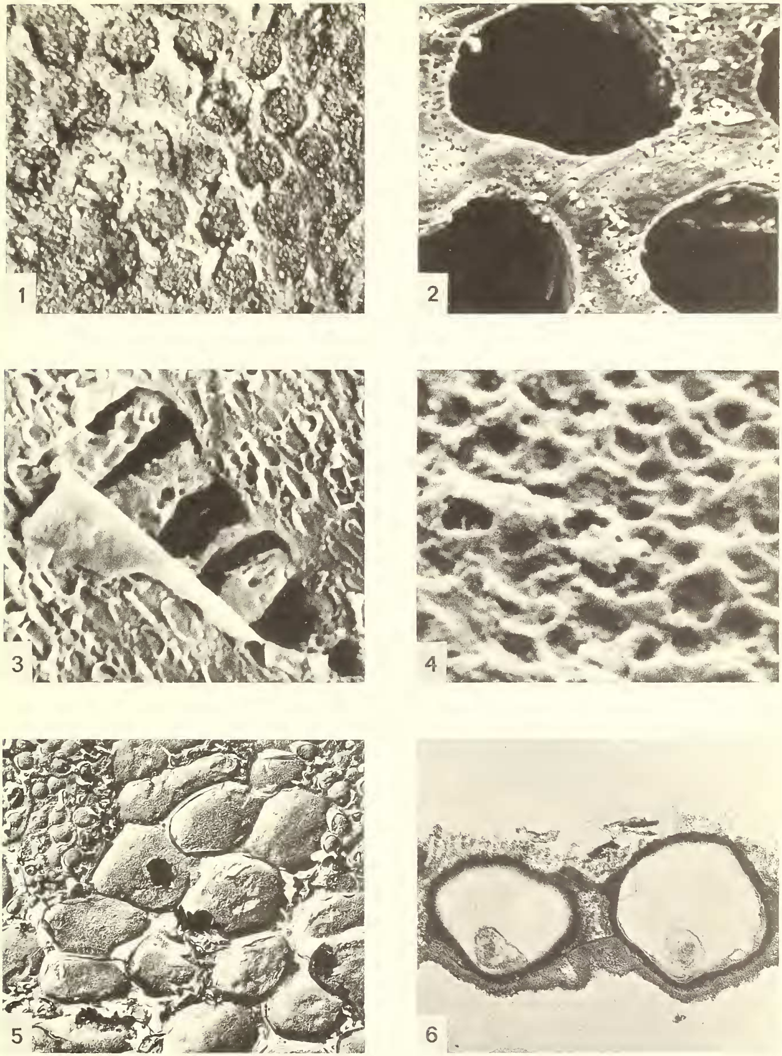
EXPLANATION OF PLATE 98

Scanning electron micrographs.

Figs. 1–3. Various aspects of protegulum of pedicle valve of *Torynelasma* sp., Arenig marly limestone, Bartoszyce, Peribaltic Depression, Poland. 1, lateral view of exterior of protegulum (×625). 2, arrangement of pits on exterior of mid-region of protegulum (×5800). 3, laminar layering of mineral parts of protegulum as seen on fracture surface more or less normal to shell with exterior to top of micrograph (×2700).

Fig. 4. Details of pit arrangement in mid-region of exterior of protegulum of topotypic pedicle valve of *Rhysotreta corrugata* Cooper (1956), mid-Ordovician Pratt Ferry Formation, Pratt Ferry, Alabama (×6500).

Figs. 5, 6. Exterior of dorsal protegulum of *Myotreta* cf. *crassa* Goryansky (1969), Arenig marly limestone, Bartoszyce, Peribaltic Depression, Poland. 5, junction between protegulum and adult (bottom left-hand corner) shell (×1300). 6, detail of pits on left lateral part of surface (×2600).



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most parts of the protegulum by recrystallization, and the rare occurrence of such pits in other Cambrian stocks has been attributed to diagenesis rather than a natural suppression of their development. There is certainly evidence of a gross recrystallization affecting even the coarser grades of pits in *Linnarssonella* where outlines indicative of hexagonal prisms have been superimposed here and there on a relict pattern of normally distributed subcircular pits.

A profound change in the distribution of pit ornamentation in early *Spondylotreta* seems, also, to be attributable to post-mortem alteration of the shell. In *S. parva* Wright (1963, p. 238) and S. *concentrica* Cooper (Pl. 100, fig. 3) from the Ashgillian and Porterfield limestones of Ireland and Alabama respectively, the pit pattern, although variably preserved in both species, is seen to be normally developed and restricted to the protegulum; but in an undescribed species of *Spondylotreta* from Tremadocian cherts of Poland, pits were found over the entire shell surface. In the specimen examined, the protegulum was ornamented by an array of pits with diameters ranging from 600 nm to 2 μm and a density count of 23 per 100 μm^2 in the mid-region (Pl. 100, fig. 4). Pits of similar size and depth, but with half the frequency distribution of those in the protegulum, also occur in the adult shell where they are limited to those exposed parts of the outer surfaces of overlapping lamellae which must have been covered by periostracum (Pl. 100, fig. 5). Despite this evidence, we believe pits on the adult shell, at least, to be solution features, because the specimens bearing them had been dissolved out of the cherts by hydrofluoric acid, and valves of *Helmersenia* and *Siphonotreta* recovered during the same operation bore similarly distributed pits (Pl. 100, fig. 6). It is still possible that the pits were formerly the sites of surface depressions which originated during shell deposition and were only enlarged during etching. This we believe to be unlikely, and we attribute the denser distribution of pits in the protegulum of the Tremadocian *Spondylotreta* to the existence of a normal array of pits, on which was superimposed a pattern of solution hollows, like those on the adult part of the shell.

No acrothelidid species has been examined but the protegulum of *Curticia*, the monotypic representative of the third acrotretacean family, is known to be pitted in the same way as that of acrotretids (Pl. 100, figs. 1, 2). It would, therefore, be surprising if the protegulum of any acrotretacean proved not to be so ornamented.

INTERPRETATION

In seeking an explanation for these distinctive arrays of pits, attention must be paid to a number of clues like the depth of the pits, their possible relationship to a restored periostracum comparable with those of living brachiopods, and to any similarities between such patterns and naturally occurring structures. In relation to their diameter and the thickness of the shell, the pits are undeniably shallow. In *Myotreta*, *Spondylotreta*, *Rhysotreta*, and *Torynelasma*, the depth of a pit appears not to be more than one-fifth of the diameter, and less than 1 μm absolutely compared with thickness of about 15 μm for the shell underlying the protegulum in adult valves. Hence the pits are not endopunctae in the sense that they were occupied by epithelial extensions persisting throughout life. Nor is it likely that they were exopunctae if the implication is that the pits were temporary sites of caecal extensions of the outer epithelium, possibly acting as food storage centres, which later became sealed off by shell deposition after withdrawal of the caeca. Terminal branches of mantle papillae penetrating the shell of living Terebratulida and Craniacea correspond to specialized microvilli and vary only narrowly in diameter immediately beneath the periostracum. In fact, the particular pattern of size variation must surely also preclude any possibility that the pits afforded accommodation for patches of specialized epithelium thereby facilitating some function of the mantle, like sensitivity to changes in light or hydrostatic pressures.

In our estimation, the distribution of pits is in itself a key to their origin. The patterns are exactly matched in bubble rafts formed on the surfaces of liquids by groups of relatively large bubbles which are partially or completely separated from one another by clusters of smaller ones. The only difference is that the surface of a raft is compositely convex, whereas that of the protegulum is compositely concave as though it were the mould of a bubble raft. The difference becomes important when one considers the nature of the periostracum that must once have covered the protegular surface. In all brachiopods, the inner sealing membrane of the periostracum acts as the seeding surface of the first apatite or calcite crystallites secreted by the epithelium; and it follows that, if the crystallites form a bubble raft mould, the inner membrane of the periostracum must have been one of the bounding surfaces of such a raft. Further inferences about the periostracum can now be made. Assuming that the vesicles making up the periostracal

EXPLANATION OF PLATE 99

Scanning electron micrographs.

Fig. 1. Details of pit arrangement in mid-region of exterior of protegulum of topotypic pedicle valve of *Conotreta depressa* Cooper (1956), mid-Ordovician Pratt Ferry Formation, Pratt Ferry, Alabama (×3900).

Fig. 2. Recrystallization superimposing a crystal fabric, as in centre of micrograph, on a pit pattern in mid-region of dorsal protegulum of *Linnarssonella girtyi* Walcott (see Bell and Ellinwood (1962)), Upper Cambrian Morgan Creek Member, Blanco Co., Texas (×2600).

Fig. 3. Distribution of pits on exterior of ventral protegulum of *Ephippelasma* sp., Llanvirn shales, Bartoszyce, Peribaltic Depression, Poland (×2600).

Fig. 4. Details of pitted ornamentation in mid-region of ventral protegulum of *Prototreta* sp., Middle Cambrian Meagher Limestone, Horseshoe Hill, Montana (×6250).

Fig. 5. Details of pitted ornamentation in mid-region of dorsal protegulum of *Scaphelasma* sp., Llanvirn marls, Ketrzyn, Peribaltic Depression, Poland (×6000).

Fig. 6. Traces of pitted ornamentation on external surface of damaged protegulum of topotypic pedicle valve of *Apsotreta expansa* Palmer (1954), Upper Cambrian Riley Formation, Llano Co., Texas (×2600).

EXPLANATION OF PLATE 100

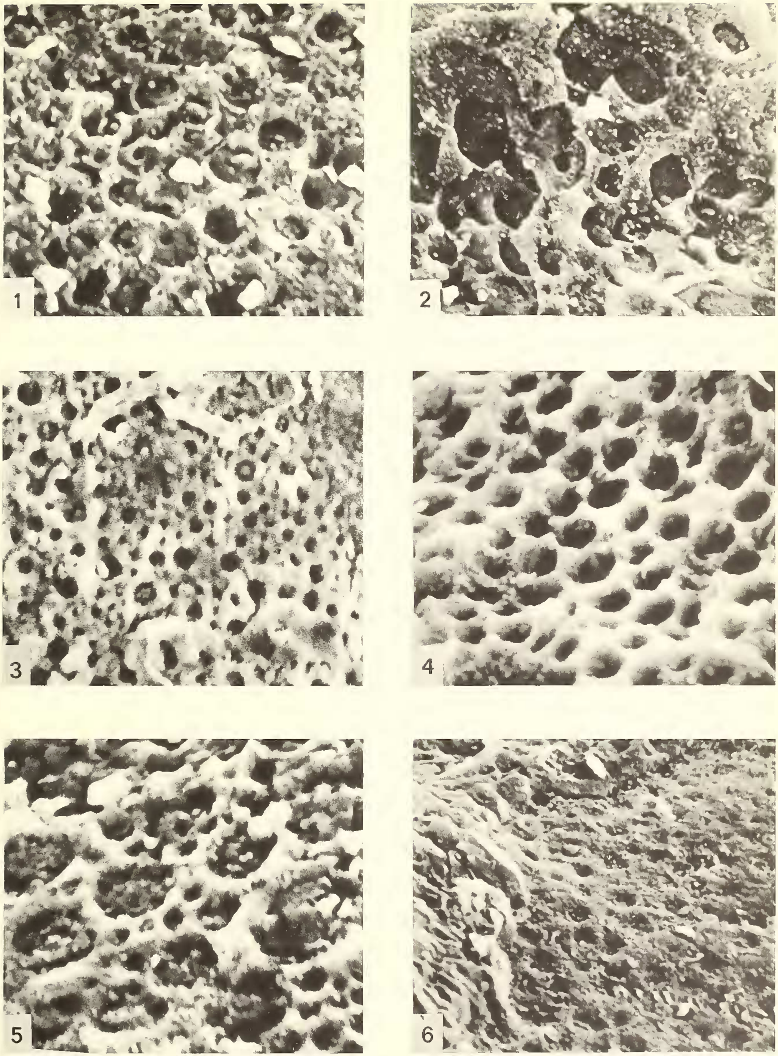
Scanning electron micrographs.

Figs. 1, 2. General view and detail of pitted ornamentation on mid-region of exterior of protegulum of topotypic brachial valve of *Curticia minuta* Bell (1941) Upper Cambrian Pilgrim Formation, Little Belt Mountain, Montana (×2500, ×6250 respectively).

Fig. 3. Distribution of pits in mid-region of exterior of protegulum of topotypic pedicle valve of *Spondylotreta concentrica* Cooper (1956), mid-Ordovician Pratt Ferry Formation, Pratt Ferry, Alabama (×6500).

Figs. 4, 5. Exterior of brachial valve of *Spondylotreta* sp., Tremadoc cherts, Wysoczki, Holy Cross Mountains, Poland. 4, distribution of pits in relation to recrystallized fabric in mid-region of protegulum (×6000). 5, presence of pits only on those parts of overlapping lamellae that make up external surface of adult shell (×1700).

Fig. 6. Distribution of external pits near margin of ventral protegulum of *Siphonotreta* cf. *acrotretomorpha* Goryansky (1969), Tremadoc cherts, Wysoczki, Holy Cross Mountains, Poland (×1300).



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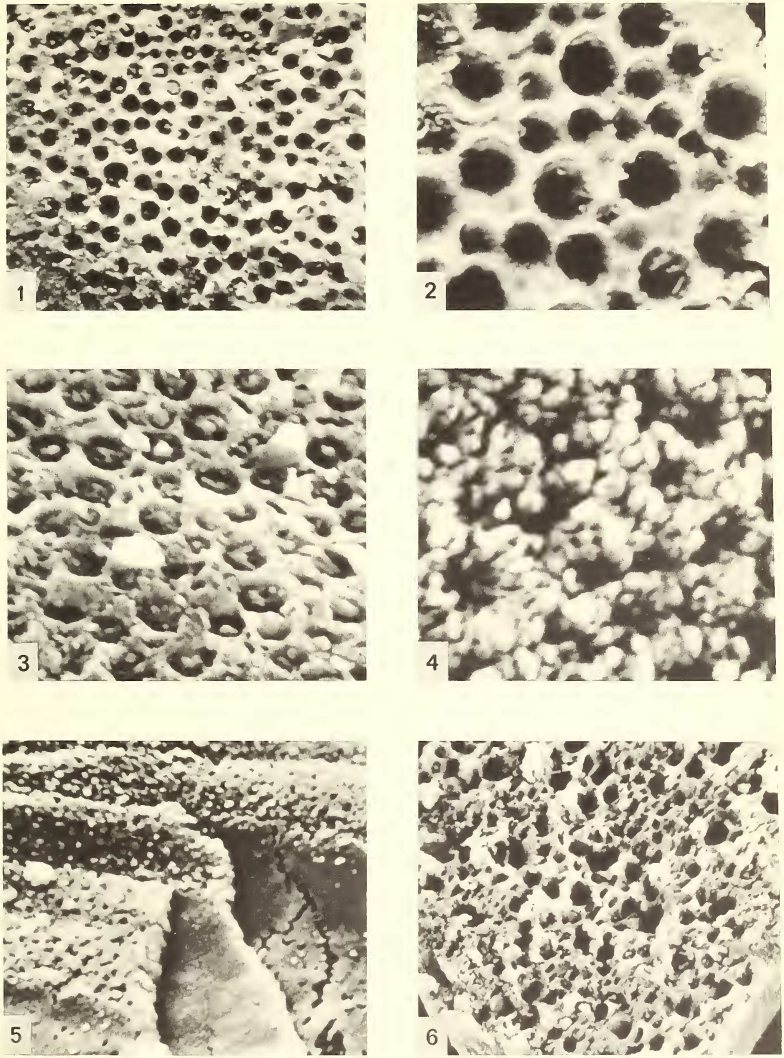

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